Specific Comments

1. Numerous tests on animals have already been conducted for these substances. Existing data sufficiently describe the relatively low toxicity of the compounds in humans and other animals, as well as the low exposures in the ambient and occupational environments.

These low molecular weight compounds, including natural gas, are such ubiquitous chemicals that, as the API states on page 3 of its own test plan: "Much is already known about how simple petroleum gases affect the body. The lighter gases (methane and ethane) are considered simple asphyxiants which means that at low concentrations they do not cause harmful effects….At the concentrations required to cause effects these gases also present an explosive hazard, so exposure to these levels is rare." It is unconscionable that the API is proposing further testing, given the existing depth of understanding of these chemicals' toxicity.

Furthermore, the API failed to perform even the most perfunctory review of the current literature and report available information. We are providing additional information that clearly shows these chemicals are not toxic in animals. Repeating tests on animals will not enhance the understanding of these petroleum gases.

In fact, the toxicity of these compounds is so low that The American Gas Association specifically requested that these compounds be exempt from the HPV program in a recent letter. This letter states, "Natural gas and all substances found therein should be deleted from the HPV list because enough information is already readily available regarding their health effects. No useful purpose would be served by performing further tests on substances whose properties are already well-understood."[4]

Moreover, when testifying about the HPV program before the U.S. House Science Subcommittee on Energy and the Environment in June 1999, Dr. Bill Sanders, Director of the EPA's Office of Pollution, Prevention, and Toxics, was questioned by Congressman Calvert about the unnecessary testing of chemicals that pose little real world risk. Dr. Sanders specifically testified that the EPA was "not requiring testing on butane."[5]

This test plan does not make use of compounds with similar structures to provide insight into the toxicity of these compounds. Because these compounds are so non-toxic, comparisons with structurally similar compounds can be used to illustrate their chemical behavior. Data from two groups of chemicals could further enlighten our understanding of natural gas toxicity: the n-alkanes in the gasoline group and crude butadiene mixtures. If n-alkanes in the higher molecular weight gasoline group do not produce toxic effects at certain endpoints, it would be entirely expected that lighter n-alkanes presented in this test plan do not produce this effect. For example, in evaluating risks from hydrocarbons at sites with petroleum-contaminated soils, reference doses of lighter alkanes are typically two to ten times higher than the reference doses for heavier alkanes.[7] Extrapolating from this trend, the risks posed by heavier, well-studied hydrocarbons in gasolines can be used as a conservative upper bound to estimate the hazards posed by the compounds in the petroleum gas group.

The butadiene test plan, submitted by the Chemical Manufacturers Association (CMA) Olefins Panel, can also provide insight into butane toxicity, as this plan presents results of repeat dose exposure studies on rats in which exposure levels reached 11,140 ppm of crude butadiene feedstock that contained 20% butanes. The conclusion of these tests was that the exposure produced "no significant adverse effects in rats." These tests are significant in that human epidemiological data and other animal testing has generally found that butadiene, with its two double bounds, is much more toxic that butane. Therefore these "reliable" GLP-level tests are consistent with the obvious observation that butane is very non-toxic, and also show that there is no synergistic effect that increases butane toxicity in these complex mixtures. In fact, the data suggest that butane reduces the toxicity of butadiene in the butadiene streams.[8]

Additional Toxicity Information on Propane

A valid acute inhalation toxicity study presented in the API's robust summary found the rat LC50 to be greater than 800,000 ppm and a rat EC50 to be 280,000 ppm. Clearly, the toxic effects of propane are negligible in rats, and these tests do not need to be repeated. To put this in perspective, the API proposes to perform acute toxicity tests at a dose of 10,600 ppm.

Guinea pigs showed sniffing and chewing movement at 22,000 to 55,000 ppm propane, with a rapidly reversible effect upon cessation of exposure. At 10,000 ppm propane, hemodynamic changes in dogs were observed, while at 33,000 ppm, decreased contractility of the heart, mean aortic pressure, stroke volume, and cardiac output were seen. In primates, 100,000 ppm propane induced some mild cardiac effects, and, at 200,000 ppm, aggravation of these parameters and respiratory depression occurred. Weak cardiac sensitization seems to be produced at 100,000 ppm propane in the mouse and 150,000 propane in the dog.[9]

In a human study, volunteers were exposed to 250 to 1,000 ppm propane and monitored for abnormal physiological responses, including pulmonary and cardiac abnormalities. No adverse effects were seen at these levels.[10]

Subchronic inhalation studies have been conducted in which monkeys were exposed to approximately 750 ppm propane for 90 consecutive days with no toxicity or abnormalities observed. Monkeys were also exposed to an aerosol spray deodorant mixture of propane and isobutane. In these studies, all animals survived and showed no changes in behavior, body weight, hematology, blood chemistry, urinalysis, electrocardiogram, or pulmonary function. Gross microscopic examination revealed no evidence of organ toxicity.[11]

Toxicity Information on Isopentane

The API did not report any toxicity data on isopentane. However, humans have been observed after exposure to up to 500 ppm, and no adverse effects were reported.[12]

Light anesthesia occurred in mice exposed to 90,000 ppm isopentane for 11.6 minutes. At higher concentrations of 110,000 and 120,000 ppm, the onset of the narcotic effect was observed within 3.9 and 2.2 minutes, respectively. The experimental LC50 after two hours of exposure was determined to be 140,000 ppm. At 120,000 ppm, dogs experienced light anesthesia.[13]

Exposure Information on Isopentane

Reported occupational exposures are much lower than OSHA or ACGIH guidelines, and orders of magnitude below the doses at which the API plans to test. For example, in an exposure study of workers at a high volume service station, employees were exposed to 0.1 to 1.3 ppm. Personal samples taken from workers in the petroleum industry indicated that 53 out of 56 outside operators were exposed to isopentane at a mean concentration of 1.64 ppm, 49 out of 49 transport drivers were exposed to a mean concentration of 3.38 ppm, and 48 out of 49 service attendants were exposed to a mean concentration of 4.55 ppm.[14,15,16]

Additional Toxicity Information on Ethane

The API only reported one acute toxicity study on dogs exposed to ethane. However, acute toxicity studies have also been conducted on guinea pigs. Guinea pigs exposed to 22,000 to 55,000 ppm of ethane for two hours showed slight signs of irregular respiration. This mild effect was reversible.[17] According to the International Labour Office's Encyclopedia of Occupational Health and Safety, humans exposed to less than 50,000 ppm of ethane

experience no systemic adverse effects.[18] An *in vitro* study was conducted that evaluated hamster embryo cells to ethane gas. Ethane did not produce any harmful effects on the cells' viability.[19]

Exposure Information on Ethane

According to the EPA's National Ambient Volatile Organic Compounds Database, the median urban concentration of ethane is 0.00915 ppm, yet the API is proposing to expose rats to 15,000 ppm.[20]

Additional Toxicity Information on Butane

The API reported acute toxicity studies in three species: rats, mice, and humans. Additional human observation has also shown that inhalation of 10,000 ppm of butane for ten minutes may result in drowsiness, but no systemic effects. Clinical information exists on people who abused butane by compulsively inhaling it.[21]

Exposure Information on Butane

Occupational studies show that even petroleum workers are exposed to relatively low levels of butane.[15,16] Environmental exposure to these chemicals are generally low, demonstrating that high production does not translate to high volume exposure. According to the EPA's Total Exposure Assessment Methodology (TEAM) studies, only 2 out of 12 personal breath samples in a highly industrial area contained n-butane.[14] According to the EPA's National Ambient Volatile Organic Compounds Database, the median urban atmospheric concentration of n-butane is 0.009174 ppm.[20]

Additional Toxicity Information on Isobutane

Monkeys were also exposed to an aerosol spray deodorant mixture of propane and isobutane. In these studies, all animals survived and showed no changes in behavior, body weight, hematology, blood chemistry, urinalysis, electrocardiogram, or pulmonary function. Gross microscopic examination revealed no evidence of organ toxicity.[22]

2. The test plan submitted by the API makes only minimal use of chemical categories.

The API proposes to subject animals to acute inhalation, repeated dose, *in vivo* genotoxicity, and reproductive/developmental tests on *all*chemicals addressed in the plan except methane. This undermines the fundamental reason for category formation: the use of sound scientific principles to reduce unnecessary tests. The application of extensive testing is even more troublesome, as animal protection organizations were assured that no acute toxicity tests would be performed when chemical categories were used, due to the "data-rich" nature of the chemicals in these categories.

3. This massive test plan ignores animal welfare concerns in other ways and specifically violates the October 1999 agreement.

The API's proposal to test all chemicals except methane on animals violates the HPV animal welfare guidance document which states that nonanimal tests should be used when available. For example, the API is proposing to conduct *in vivo* genotoxicity tests on all chemicals within the category except methane when *in vitro* tests exist and have already been conducted and presented in the Robust Summary. *In vitro* tests for mutagenicity and chromosomal aberration (OECD Test Guidelines 471 and 473) preclude the need for any *in vivo* genetic toxicity tests. Furthermore, the API proposes to do a reproductive/developmental test and a developmental test, when the combined test (OECD Test Guidelines 421) is sufficient.

4. The test protocol does not use "thoughtful toxicology."

Acute toxicity tests will be conducted at half the lower explosive limit (LEL), which the API admits is below the level at which an effect in humans or other animals is seen. For example, the API is proposing to perform toxicity tests on propane, a substance for which human occupational guidelines exist. Propane has already been tested and found to have a rat LC50 greater than 800,000 ppm. Despite this evidence that toxic effects are negligible in rats, the API is proposing to do additional tests at half the LEL, which is 10,000 ppm. This concentration is 80 times lower than the rat LC50 presented in its robust summary. Clearly, these experiments are unnecessary and costly both financially and in terms of animal life. Rat and dog LC50s for isobutane were found to be 570,000 ppm and 70,000 ppm, respectively. The API is proposing to test isobutane in rats again at half the LEL, which is 9,000 ppm, 60 times lower than the reported LC50. Moreover, human experiments have been conducted on isobutane. The API reports a study in which human volunteers were exposed to isobutane in a controlled chamber environment to concentrations of 250 to 1,000 ppm. No symptoms were observed.

The testing protocol is highly flawed and repeats existing, readily available information. The proposed tests will subject animals to gas levels that are known to have no effect. What value can possibly be gained from these experiments, especially since human data exist? Humans are exposed to low levels of these gases in occupational and home environments. Since three of these chemicals are regulated by OSHA, monitoring of workers is necessarily taking place, and there is ample opportunity for human observation.

The API should employ a more thoughtful approach to understanding the systematic toxicity of the alkane compounds in the group, by considering the toxicity of well-studied higher molecular weight alkanes and alkenes found in petroleum products. For example, if C6 to C10 n-alkanes have been shown to have minimal reproductive/developmental effects, butane and pentane also should not have these effects. In the alkanes with eight or fewer carbons, toxicity is generally inversely proportional to molecular weight.[7] Therefore, the better characterized n-alkanes in gasoline should be used to provide an upper bound on the toxic effect of butanes and pentanes. Applying toxicity data from C6 to C8 n-alkanes to understand the toxicity of C4 and C5 n-alkanes would constitute a straightforward application of structure-activity relationships.

In addition, test plans on two other chemicals shed light on the toxicity of the petroleum gases: butadiene and the C5 noncyclics category. The butadiene plan, submitted by the CMA Olefins Panel, provides information on the toxicity of complex mixtures of these gases, which also contain the more toxic compound butadiene, and the C5 gases test plan provides some information on n-alkanes. The data in these plans show that C4 to C6 compounds with fewer double bonds are generally less toxic than compounds with more double bonds. Given that the compounds in this petroleum gas group are all alkanes with similar numbers of carbons and no double bonds, the butadiene and C5 plans confirm that the toxicity of the petroleum gas compounds is consistently low at non-explosive levels.

The few compounds that the API has selected are the shortest chain, lowest molecular weight, and the most non-toxic substances found in petroleum gases. People are exposed to these chemicals at very low levels—concentrations that are known to have no adverse effects in humans. Animals have already been subjected to numerous toxicity tests, and concentrations causing any harm are extremely high. The API proposes to test the chemicals again in animals, at levels much lower than LC50s obtained in prior studies.

This entire test plan is ill-conceived, unnecessary, and will do nothing to advance public health. Extensive human and animal data have already been collected on exposures to these compounds, and to better advance the public's understanding of how these chemicals may impact human health, further exposure and epidemiological studies should be conducted. It is astounding that the API has put together such a shoddy test plan, and there is absolutely no need to subject animals to further toxicity tests on these relatively non-toxic, well-characterized chemicals.

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